AC Cinnamon Liposome Efficacy Data

Code: 16098
INCI Name: Water & Cinnamomum Cassia Bark Extract & Phospholipids
CAS #: 7732-18-5 & 84961-46-6 & 123465-35-0
EINECS #: 231-791-2 & 284-635-0 & N/A

Name of Study	Results
Cellular Viability Assay	**AC Cinnamon Liposome** exhibited positive results by increasing cell metabolism. The increase in fluorescent signal indicates an increase in cellular metabolism and viability post **AC Cinnamon Liposome** treatment. For these reasons, we can assume **AC Cinnamon Liposome** is suitable for cosmetic applications designed to increase cell viability and metabolism.
Tyrosinase Inhibition Assay	2.0% **AC Cinnamon Liposome** inhibits tyrosinase activity by 40.6% and 5% **AC Cinnamon Liposome** inhibits tyrosinase activity by 65.4%. For this reason, **AC Cinnamon Liposome** is ideal for lightening the skin to promote an even and healthy looking skin tone.
In-vivo Skin Lightening Study	2.0% **AC Cinnamon Liposome** has significant impact on melanin levels. **AC Cinnamon Liposome** reduced melanin levels by 10% and by 12% when compared to the untreated control and to the base cream, respectively.
ORAC Assay	**AC Cinnamon Liposome** exhibited similar antioxidant properties to our Trolox® positive controls. The antioxidant capacity of **AC Cinnamon Liposome** increased as the concentration increased, as a result we can assure that its ability to minimize oxidative stress is dose dependent

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Cellular Viability Assay Analysis

Tradename: AC Cinnamon Liposome

Code: 16098

CAS #: 7732-18-5 & 84961-46-6 & 123465-35-0

Test Request Form #: 468

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Erica Segura*
Principle Investigator: *Meghan Darley*

Test Performed:
Cellular Viability Assay

Introduction

The cellular viability assay is useful for quantitatively measuring cell-mediated cytotoxicity, cell proliferation and mitochondrial metabolic activity. Increased metabolism in a cell indicates ample cellular respiration and adenosine triphosphate (ATP) production. ATP is the molecular energy of cells and is required in basic cell function and signal transduction. A decrease is ATP levels indicates cytotoxicity and decreased cell function while an increase in ATP levels indicates healthy cells.

The cellular viability assay was conducted to assess the ability of **AC Cinnamon Liposome** to increase cellular metabolic activity in cultured dermal fibroblasts.

Assay Principle

The assay utilizes a nonfluorescent dye, resazurin, which is converted to a fluorescent dye, resorufin, in response to chemical reduction of growth medium from cell growth and by respiring mitochondria. Healthy cells that are in a proliferative state will be able to easily convert resazurin into resorufin without harming the cells. This method is a more sensitive assay than other commonly used mitochondrial reductase dyes such as MTT. An increase in the signal generated by resazurin-conversion is indicative of a proliferative cellular state.

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Materials

A.	**Kit:**	PrestoBlue™ Cell Viability Reagent (Invitrogen, A13261)
B.	**Incubation Conditions:**	37°C at 5% CO_2 and 95% relative humidity (RH)
C.	**Equipment:**	Forma humidified incubator; ESCO biosafety laminar flow hood; Light microscope; Pipettes
D.	**Cell Line:**	Normal Human Dermal Fibroblasts (NHDF) (Lonza; CC-2511)
E.	**Media/Buffers:**	Dulbecco's Modified Eagle Medium (DMEM); Penicillin-Streptomycin (50U-50mg/mL); Fetal Bovine Serum (FBS); Phosphate Buffered Saline (PBS)
F.	**Culture Plate:**	Falcon flat bottom 96-well tissue culture treated plates
G.	**Reagents:**	PrestoBlue™ reagent (10X)
H.	**Other:**	Sterile disposable pipette tips

Methods

Human dermal fibroblasts were seeded into 96-well tissue culture plates and allowed to grow to confluency in complete DMEM. A 10-fold serial dilution was performed resulting in **AC Cinnamon Liposome** concentrations on 1%, 0.1%, and 0.01% in complete DMEM and incubated with fibroblasts for 24 hours.

Ten microliters of viability reagent was added to 90µL of cell culture media in culture wells.

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Results

The data obtained from this study met criteria for a valid assay and the controls performed as anticipated.

AC Cinnamon Liposome exhibited positive effects on cellular metabolism compared to the control.

Cellular metabolism results are expressed as a percentage of the control.

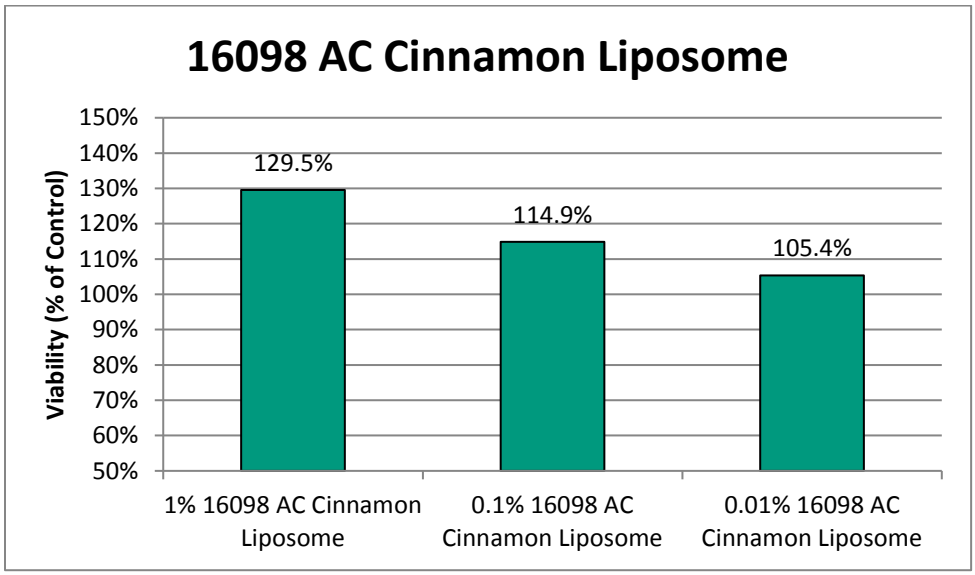

Figure 1: Cellular Metabolism of **AC Cinnamon Liposome**-treated fibroblasts expressed in terms of percent of control.

Discussion

As shown in figure 1, **AC Cinnamon Liposome** exhibited positive results by increasing cell metabolism. The increase in fluorescent signal indicates an increase in cellular metabolism and viability post **AC Cinnamon Liposome** treatment. For these reasons, we can assume **AC Cinnamon Liposome** is suitable for cosmetic applications designed to increase cell viability and metabolism.

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AC Cinnamon Liposome Tyrosinase Inhibition Study

Code: 16098
INCI Name: Water & Cinnamomum Cassia Bark Extract & Phospholipids
Suggested Use Levels: 1.0-10.0%

Abstract

The purpose of this *in-vitro* study was to determine the effects of **AC CInnamon Liposome** on tyrosinase inhibition. Tyrosinase is an enzyme that plays a major role in melanogenesis, or the synthesis and expression of melanin within the skin. Studies were conducted on isolated mushroom tyrosinase utilizing UV-Visible Spectrophotometry. The results indicate that 2% **AC Cinnamon Liposome** is capable of inhibiting the activity of tyrosincase by 40.6% and 5% **AC Cinnamon Liposome** inhibits tyrosinase activity by 65.4%.

Materials and Methods

Mushroom tyrosinase was isolated and the below in-vvitro test was conducted using a water blank (negative control), 1% Kojic Acid (positive control), 2% and 5% **AC Cinnamon Liposome**. Inhibition was measured after 30 minutes using UV/Vis Spectrophotometer (Shimadzu UV-1601). The specifications for the use of the spectrophotometer are the following:

Temperature-25 degrees Celsius, pH-6.5, Absorbance 280nm and path length-1cm.

A reaction mixture was prepared by pipetting the following reagents in to a clean container:

Deionized water-9.00 ml, reagent A-10.0 ml of potassium phosphate buffer 50mM, Reagent B-10.0 ml of tyrosine solution-1 mM.

Results

The solution was mixed and the pH was adjusted to 6.5 using 1 M HCl. The mixture was then pipetted into quartz cuvettes. This was equilibrated to 25 degrees Celsius. A280 was monitored until constant. The reagents were added as follows:

	Test	Blank
Reaction Mixture	2.90	2.90ml
Reagent A	-	.10ml
Reagent B	0.10	-

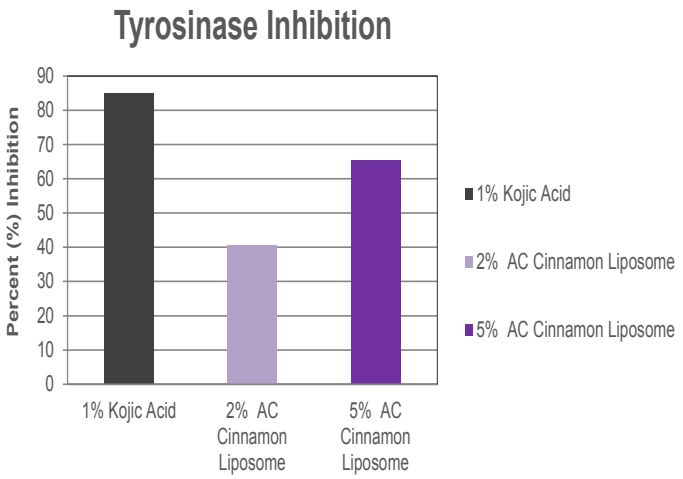

Figure 1. Percent (%) tyrosinase inhibition of test materials.

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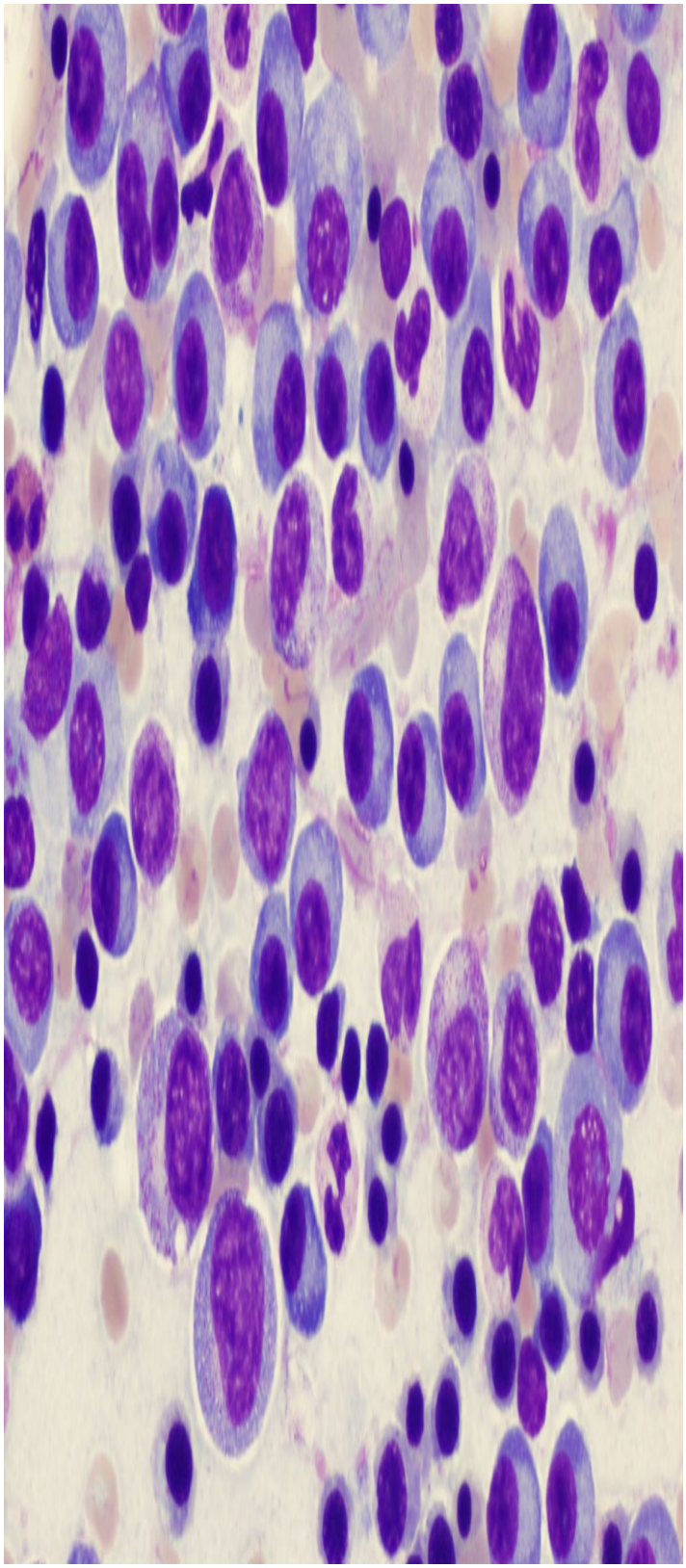
Discussion

Based on the results, 2% **AC Cinnamon Liposome** inhibits tyrosinase activity by 40.6% and 5% **AC Cinnamon Liposome** inhibits tyrosinase activity by 65.4%. For this reason, **AC Cinnamon Liposome** is ideal for lightening the skin to promote an even and healthy looking skin tone.



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AC Cinnamon Liposome *In-vivo* Skin Lightening Study

Code: 16098
INCI Name: Water & Cinnamomum Cassia Bark Extract & Phospholipids
Suggested Use Levels: 1.0-10.0%

Abstract

AC Cinnamon Liposome was developed to reduce hyperpigmentation to even the skin tone. The natural components within cinnamomum cassia bark extract are capable of chelating copper to prevent tyrosinase activity. Without tyrosinase, melanogensis can not be intiated therefore melanin can not be produced.

The objective of this study was to determine whether or not **AC Cinnamon Liposome** is capable of reducing hyper-pigmentation to even the skin tone. Based on our findings **AC Cinnamon Liposome** is capable of significantly reducing areas of hyperpigmentation in order to even the skin tone.

Materials and Methods

The study was conducted with 5 M/F subjects between the ages of 24-33. Subjects applied 2 mg of a base lotion containing 2% **AC Cinnamon Liposome** to their volar forearms twice daily for 14 days.

Equipment used was the DermaLab Combo Skin Lab (color detecting chip) equipped with a pigmentation probe. Two high intensity white LED lights provide illumination, and the proves color sensor records the readings.

Melanin is calculated as an index to provide pigmentation level and reaction to irritants. The higher the index, the higher the pigmentation level.

Results

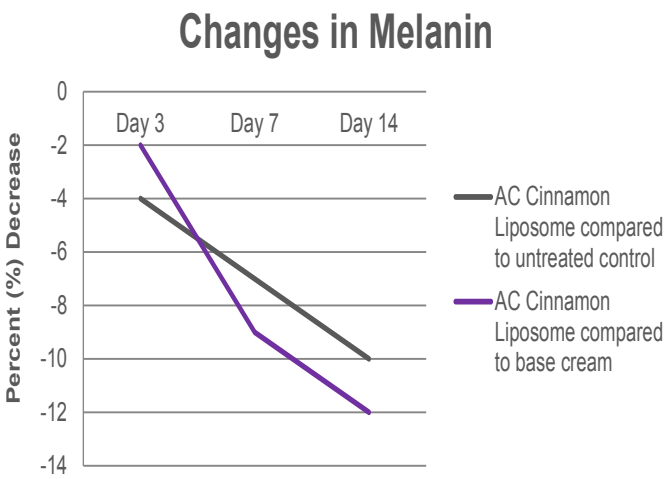

Figure 1. Changes in melanin of 2% **AC Cinnamon Liposome** compared to the base cream and untreated control.

Discussion

Based on the results, 2% **AC Cinnamon Liposome** has significant impact on melanin levels. **AC Cinnamon Liposome** reduced melanin levels by 10% and by 12& when compared to the untreated control and to the base cream, respectively.

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Oxygen Radical Absorbance Capacity (ORAC) Assay

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Tradename: AC Cinnamon Liposome

Code: 16098

CAS #: 7732-18-5 & 84961-46-6 & 123465-35-0

Test Request Form #: 35

Lot #: NC120618-I

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Erica Segura*
Principle Investigator: *Meghan Darley*

Test Performed:
Oxygen Radical Absorbance Capacity (ORAC) Assay

Introduction

Reactive oxygen species (ROS) are generated by normal cellular processes, environmental stresses, and UV irradiation. ROS are dangerous to cellular structures and functional molecules (i.e DNA, proteins, lipids) as they act as strong oxidizing agents or free radicals. The oxygen radical absorbance capacity (ORAC) assay is a standard method used to assess antioxidant capacity of physiological fluids, foods, beverages, and natural products. The assay quantitatively measures a sample's ability to quench free radicals that have the potential to react with and damage cellular components.

Oxygen Radical Absorbance Capacity (ORAC) assay was conducted to assess the antioxidant capacity of **AC Cinnamon Liposome**.

Assay Principle

This assay is based upon the effect of peroxyl radicals generated from the thermal decomposition of 2, 2'-azobis-2-methyl-propanimidamide dihydrochloride (AAPH) on the signal intensity from the fluorescent probe, fluorescein, in the presence of an oxygen radical absorbing substance. The degree of change is indicative of the amount of radical damage and the presence of antioxidants results in an inhibition in the free radical damage to the fluorescein. The antioxidant protection of the sample can be calculated by comparing it to a set of known standards. Trolox®, a water soluble vitamin E analog, with known antioxidant capabilities is used in this ORAC assay as the standard for measuring the antioxidant capacity of unknown substances. ORAC values, expressed in µM of Trolox® equivalents (TE), are calculated using the area under the curves (AUC) of the test product, Trolox®, and the control materials. Trolox equivalency is used as the benchmark for antioxidant capacity of mixtures since it is difficult to measure individual components.

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Materials

A. Equipment:	Synergy H1 Microplate reader (BioTek Instuments, Winooski, VT); Gen5 software (BioTek Instuments, Winooski, VT); Pipettes
B. Buffers:	75mM Potassium Phosphate (pH 7.4); Deionized H_2O
C. Reagents:	2,2′-Azobis(2-methylpropionamidine) dihydrochloride (AAPH) (153mM); 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox®); Fluorescein Sodium Salt (4nM)
D. Preparation:	Pre-heat (37°C) Synergy H1 Microplate reader; Prepare Trolox® standards, sample dilutions, fluorescein solution, and AAPH.
E. Microtitre Plates:	Corning 96 Well Black Side/Clear Bottom Microplates

Methods

Solutions of **AC Cinnamon Liposome** and Trolox® (positive control) were prepared in 75mM potassium phosphate buffer. Materials were prepared at three different concentrations/dilutions. Trolox® was used as a reference for antioxidant capacity and prepared at a concentrations ranging from 12.5µM to 200µM in 75mM potassium phosphate buffer.

For the ORAC assay, 25µL of test material and Trolox® were combined with 150µL of fluorescein in 75mM potassium phosphate buffer and incubated in the Synergy HT Microplate reader at 37˚C for 30 minutes. At the end of the incubation period, 25µL of AAPH were pipetted into each well. Fluorescent measurements were then taken every 2 minutes for approximately 2 hours at an excitation wavelength of 485nm and an emissions wavelength of 520nm.

The AUC and Net AUC values of the standards and samples were determined using Gen5 2.0 Data Reduction Software using the below equations:

$$AUC = 0.5 + \frac{R2}{R1} + \frac{R3}{R1} + \frac{R4}{R1} + \cdots + \frac{Rn}{R1} \rightarrow Where\ R\ is\ fluorescence\ reading$$

$$Net\ AUC = AUC_{sample} - AUC_{blank}$$

The standard curve was obtained by plotting the Net AUC of different Trolox® concentrations against their concentration. ORAC values of samples were then calculated automatically using the Gen5 software to interpolate the sample's Net AUC values against the Trolox® standard curve. ORAC measurements for the test material were expressed in micro moles Trolox® equivalents per mL (µMTE), where 1 ORAC unit is equal to 1 µMTE.

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Results

AC Cinnamon Liposome began showing antioxidant activity at 0.05%.

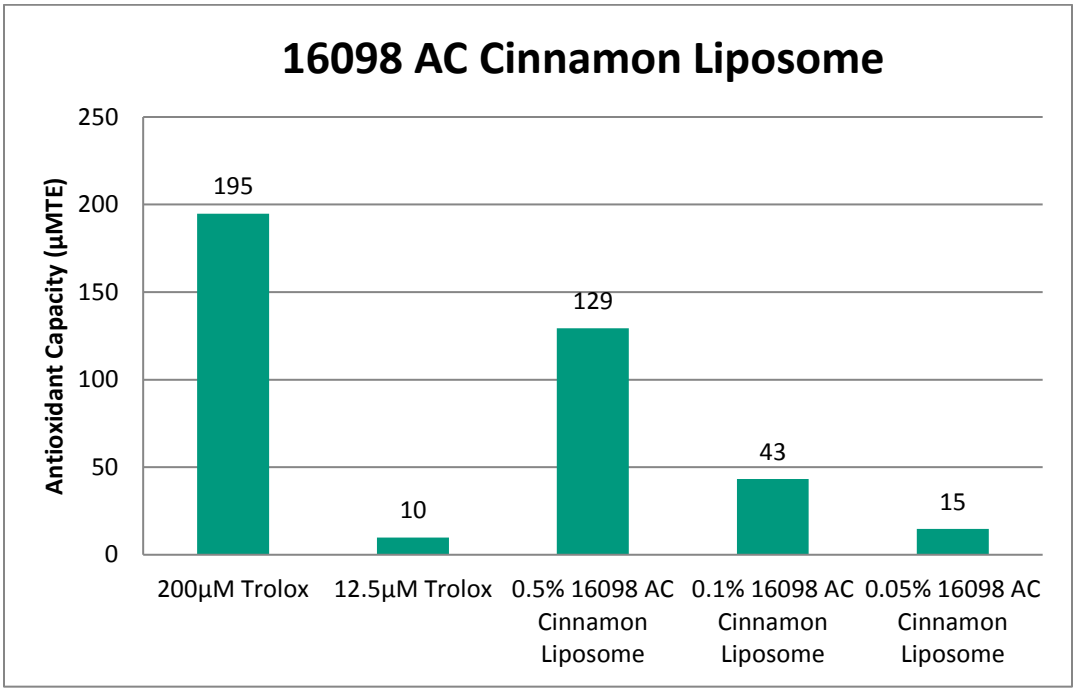

Figure 1: Antioxidant capacities

Discussion

As shown in figure 1, **AC Cinnamon Liposome** exhibited similar antioxidant properties to our Trolox positive controls. The antioxidant capacity of **AC Cinnamon Liposome** increased as the concentration increased, as a result we can assure that its ability to minimize oxidative stress is dose dependent.

AC Cinnamon Liposome was designed to provide skin lightening properties, however with the present study we can confirm that this unique ingredient is not only capable of providing functional benefits but it is also capable of providing potent antioxidant benefits when added to cosmetic and personal care applications.